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PRACTICAL METHODOLOGY OF EVALUATION OF MORTALITY CURVES AND DETECTION OF AGING-RELATED INTERVENTIONS

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ABSTRACT

A practical and simple method of direct proof of occurrence of changes in the aging rate is suggested. The aging rate is defined here according to the pacemaker concept of the control of aging as the rate of pace of the inner aging clock. The principle of the method consists of the analysis of relationships between mortality curves. The methodology makes it possible to distinguish the changes in mortality caused by direct intervention in the basic mechanism of the control of aging processes (the pacemaker) from changes caused by intervention in other systems of an organism. As mortality curves are often difficult to obtain directly in experimental gerontology, a method of transformation of survival data into mortality curves is demonstrated. The general purpose of this study is to derive more information from experimental data and from demographic studies, and contribute to a more exact methodology of verification in gerontology.

INTRODUCTION

To determine whether and how the aging rate is changed by experimental interventions is often a crucial point for interpretation of the results of experiments and conclusions derived from population studies. Such methodology is important for verification of theories and hypotheses in gerontology. Unambiguous verification is the most crucial unresolved problem in this science. The existence of numerous hypotheses and theories of aging is also in part the result of this situation.

The currently applied methods, mainly based on measurement and assessment of changes in biomarkers of aging or on the analysis of mean and maximum life span, are essentially indirect. Similarly, indirect information is provided by recent methods of assessment of biological age, as they are in principle batteries of selected biomarkers. Despite considerable effort, a practical and reliable methodology of determining the state of an organism with respect to real or biological age is still an open problem.

Nevertheless, the direct proof of occurrence of the changes of the aging process may be derived from analysis of the incidence of death, specifically, from mortality curves. Simply stated, aging results in the growth of the risk of death, and the quantity describing this risk is mortality.

In principle, mortality curves contain information which makes it possible to detect intervention into systems controlling the aging rate. Sacher (1), Failla (2), Strehler (3,4), Doubal (5), Riggs (6), Lestienne (7), Piantanelli (8) and many others analyzed laws governing the dependency of mortality on age. In our previous paper (9) we analyzed a simple theoretical model of aging based on the concept of existence of an inner clock of aging — in other words, on the existence of a pacemaker system which controls the basic "setup" of the rate of the aging processes. There is strong evidence for the existence of the pacemaker (4, 8, 9). The idea that aging is controlled by a single pacemaker is also strongly supported by the fact that Gompertz law holds for populations living in a wide range of environmental conditions (4, 9). Accordingly, the aging rate and aging rate changes are treated here as the rate of pace of the inner aging clock and changes in this pace. The methodology suggested in the present paper, based on analysis of mortality (and survival) curves, applies the results of cited work (9) and makes it possible to detect interventions into the pacemaker.

In our previous paper (9) we suggested methodology of elimination of this ambiguity. It is based on the fact that mortality curves in Gompertz period are determined by two parameters: the mortality in arbitrary chosen time (e.g., initial mortality) and the slope of the curve. According to our analysis, intervention into the aging process modifies these two parameters specifically (see *Interpretation of Changes in Gompertzian Parameters*). Consequently, by extracting and comparing mortality curves, we are able to determine whether the aging rate was modified.

Mortality curves are frequently used in study of aging of human populations. The application of analysis of mortality curves is less common in experimental research. Nevertheless, Hosokawa (11) and Johnson (12) supported conclusions of their works by comparison of mortality curves. The apparent necessity of large experimental groups for acquiring mortality curves seemingly represents the main practical limitation. However, this problem may be easily overcome in the case of Gompertz-type mortality curves, as the next section shows.

METHOD

Transformation of Survival Data into Mortality Curves

Gompertz law of exponential growth of mortality holds for all human populations in industrial countries as well as for the majority of laboratory mammals and even for many other species of laboratory animals (see Discussion):

$$R(t) = R_0 e^{k(t-t_0)}$$

where R(t) is (the rate of) mortality, R_0 mortality at time t_0, t is time or age.

For mortality, the following formula holds generally:

$$R(t) = -\frac{1}{P(t)}\frac{dP(t)}{dt}$$

where $P(t)$ is the probability of survival, which is in practice estimated by either experimental or natural (demographic) survival curve $N(t)$:

$$P(t) = \frac{N(t)}{N_0}$$

($N(t)$, N_0 is the number of individuals living at time t, t_0, respectively).

The above formulae may be connected and the resulting differential equation

$$R_0 e^{k(t-t_0)} = -\frac{1}{P(t)}\frac{dP(t)}{dt}$$

has (for condition $P(t_0)=1$, i.e. $N(t_0)=N_0$) the unique solution

$$P(t) = e^{-\frac{R_0}{k}(e^{k(t-t_0)}-1)}$$

It follows from the last formula that if the data of survival $N(t)$ are available, estimates of parameters R_0 and k of the mortality curves may be obtained. Since linearization of function $P(t)$ is not possible, procedures of nonlinear regression are suitable for this purpose. Since nonlinear regression methods are available with common statistical software equipment for the PC, the transformation of survival data into mortality curves is an easy task.

In principle, it is possible to obtain the parameters of Gompertz curve by directly calculating mortalities from survival data and then by applying regression to them. The disadvantage of methods based on this possibility was shown in Wilson's paper (13). The comparison of four different methods presented in the quoted paper leads to the conclusion that nonlinear regression gives the most precise and reliable evaluation of the parameters.

Examples

To illustrate applications of the transformation, two cases of experimental results published recently in the present journal are used as examples. Although the original articles do not contain numerical values of survival, the necessary values were determined from the published graphs only.

The first case, based on (14), analyzes the effect of temperature to the survival of Drosophila melanogaster female adults. Figure 1 shows the original data (points) and corresponding theoretical "Gompertzian" survival curves determined by means of nonlinear regression (we used SOLO statistical software). By means of this regression, not only were the values R_0, k obtained, but also their standard errors, s_k, which are substantial for the evaluation of statistical significance of the results (see Discussion). Figure 2 shows one possible standard way of representation of all these results. The thick straight lines represent the natural logarithm of mortality determined by parameters; in $R_{0,k}$, the dashed lines represent their "limits" determined by parameters S_R, S_K.

The other case is based on (15), which concerns the effect of adding 2-mercaptoethylamine hydrochloride (2-MEA) to the diet of mice. The same method of evaluation was used here as before, with only one modification. While in (14) 100% survival was postulated at zero age (i.e., $t_0 = 0$ in the evaluated expression), here it was at the age of 15 weeks ($t_0 = 15$). This partially explains the disproportionate differences in the shape of the theoretical survival curves in Figures 1 and 3, but it has no relation to the differences in resulting straight lines (Figures. 2 and 4, see the next paragraph and Discussion).

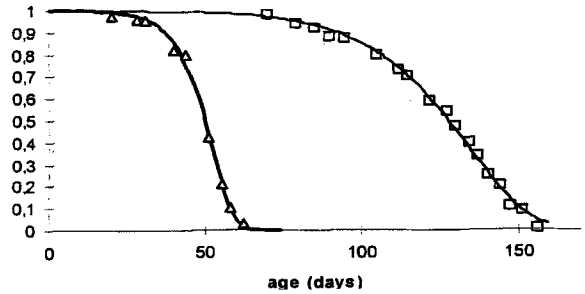

Figure 1. Survival curves demonstrating the effect of temperature (20° C — thin line, 28°C — thick line) to the survival of adult D. melanogaster females.

Figure 2. Gompertzian lines corresponding with survival curves in Figure 1. Values of constants obtained by nonlinear regression are shown in Table 1.

Interpretation of Changes in Gompertzian Parameters

In our previous work (9), we analyzed relationships between parameters of mortality curves and changes in the inner setup of aging for systems with pacemaker mechanisms of aging control. We showed there that effective intervention into the pacemaker (i.e., the rate

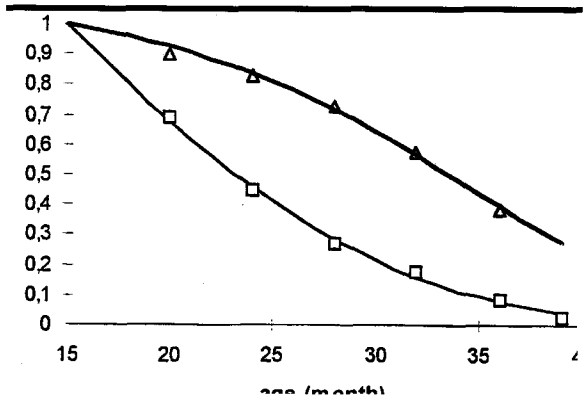

Figure 3. Survival curves demonstrating the effect of adding 2-MEA to the diet of mice (0% MEA – thin line; 1% MEA – thick line).

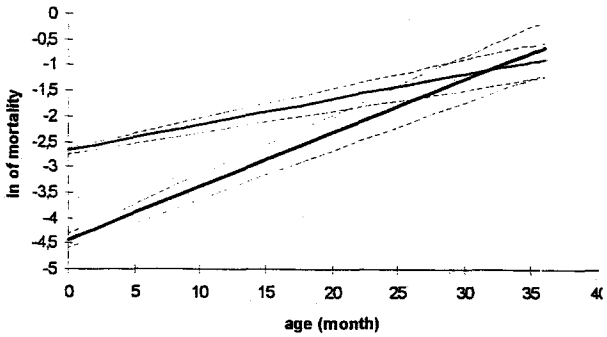

Figure 4. Gompertzian lines corresponding with survival curves in Figure 3. Values of constants obtained by nonlinear regression are shown in Table 2.

Table 1. Values of constants used in Figure 3.

	k	s_k	l_0	s_l
20°C (thin line)	0.0532	0.0009	4.14E-05	4.80E-06
28°C (thick line)	0.149	0.0053	6.22E-05	1.66E-05

Table 2. Values of constants used in Figure 4.

	k	s_k	λ_0	s_λ
0% MEA	0.0497	0.0077	0.0686	0.0041
1% MEA	0.105	0.011	0.0119	0.0015

of aging) specifically modifies the parameters of mortality curves. Let us briefly review the basic concepts and conclusions of the cited paper in a slightly modified and simplified form.

The model of aging is based on the general system theory and describes aging in "Gompertz population," i.e., in a population where the logarithm of mortality $y(t) = \ln R(t)$ is a linear function of time in a certain "Gompertz range" $t_o < t < t_1$:

$$y(t) = k\,(t - t_o) + y(t_o). \quad [1]$$

The population is supposed to be homogenous in the sense that the dispositions of individuals to the aging are equivalent. In this case, each individual represents a stochastic system with the equal probability $P(t)$ of living until the age t. In other words, the time course of $P(t)$ is the theoretical course of the survival curve of that population, which is in one-to-one correspondence with a course of mortality for definite values of parameters k, $y(t_o)$.

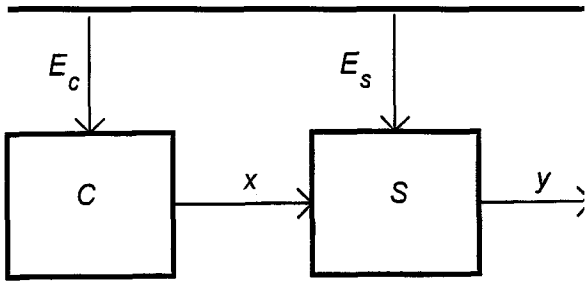

Figure 5. The structure of the model of aging. C – the pacemaker ("clock") subsystem; S – the subsystem representing the organism except the pacemaker; E_c, E_s – the inputs determined by the environment (variables x, y – see the text).

The structure of the model is shown in Figure 5. It consists of only two subsystems: the pacemaker ("clock") C and subsystem S representing the remainder of the organism except for the pacemaker itself. The two inputs E_c, E_s represent the effects of a given type of environment to the pacemaker or to the rest of the organism, respectively. The value of variable x represents the actual "state of the clock," the pacemaker controlled constituent of the age of the organism. Output variable y is the logarithm of actual mortality as mentioned above.

No changes in the effects E_c, E_s of environment to population are supposed during the time period under study. Hence, there are no changes in parameters determining the time course of variables x, y during this period. The fact that the output $y(t)$ is linear implies that the course of the "state of the clock" is a linearizable (e.g., exponential) function of time. It is only a matter of convenience to express $x(t)$ formally as linear function

$$x(t) = a_c\,(t - t_o) + b_c \quad [2]$$

where the constants a_c, b_c are determined only by (genetically determined) properties of the pacemaker itself and by the input E_c. In fact, constant a_c itself expresses the "speed" of the pacemaker, i.e., the aging rate, while the initial "state of the clock" is determined by constant $b_c = x(t_o)$.

Finally, the linear time course of output $y(t)$ is determined by three factors: the initial state of the organism S_o, the constant effect of environment E_s and the input variable x of the subsystem S. As both functions (t), $y(t)$ are linear functions of time, the interrelation must also be linear.

$$y(t) = a_s x(t) + b_s.$$

Substitution of [2] into this equation gives:

$$y(t) = a_s a_c\,(t - t_o) + a_s b_c + b_s \quad [3]$$

and the comparison with equation [1] gives:

$$k = a_s a_c \qquad y(t_o) = a_s b_c + b_s \quad [4]$$

This result leads to the following conclusions: All environmental parameters except a_c cause a change in $y(t_o)$, i.e., in vertical displacement of the Gompertz straight line; parameter a_c, changes only the slope k. If parameter b_c, is not changed by environmental effects, only parameters a_s, b_s can affect $y(t_o)$. In other words:

1. *Changes exclusively in the slope of the Gompertz line can be caused only by changes of "speed" of the pacemaker.*

2. *Vertical displacements of the initial point $y(t_o)$ of the Gompertz line are not caused by affecting the "speed" of the pacemaker, i.e., by affecting the aging rate.*

Only the parameter a_s affects both k, $y(t_o)$. Let us compare two Gompertz lines differing in values $a_{s1} \neq a_{s2}$ only. The point of intersection of the lines is then determined by equation $y_1(t_i) = y_2(t_i)$, i.e.:

$$a_{s1}[a_c(t_i - t_o) + b_c] = a_{s2}[a_c(t_i - t_o) + b_c]$$

which (for $a_{s1} \neq a_{s2}$) has the unique solution:

$$[a_c(t_i - t_o) + b_c] = 0,$$

i.e.,

$$t_i = t_o - b_c / a_c$$

This solution implies:

3. *The point of intersection $[t_i, y(t_i)]$ of Gomptertz lines does not depend on changes of the environmental parameter a_s but strongly depends on the parameters of the pacemaker.*

DISCUSSION

First, let us briefly discuss point 3 of the previous section. It is well known that changes in Gompertz lines for human populations result generally in convergent lines with a strong tendency to intersect in one fixed point for many years (e.g., as Riggs showed (16), in the period 1900-1986 for the US population). The above described model offers both explanation and interpretation of this phenomenon: all the changes in environment in lifestyle and medical care during this long period did not affect the basic mechanism of aging — the pacemaker.

The results of analysis of the model may be used for evaluation of experimental mortality curves only if the basic presumption is fulfilled: no effective changes are allowed in environmental conditions during the Gompertz period. This was granted in both examples cited here by keeping the experimental and control populations in different but unchanged conditions.

In practice, evaluation of changes in coefficients R_o, k must be used for the decision on the statistical significance of conclusions based on the theoretical results of the last section. To prove divergence of Gompertzian lines (apparent in the first example, Figure 2), two inequalities must be statistically proved:

$$k_1 > k_2 \text{ and not } (R_{01} < R_{02})$$

where subscript 1 marks parameters of the upper straight line in Fig. 2.

Similarly, to prove convergence of lines (as seen in the second example, Figure 4), inequalities

$$k_1 < k_2 \text{ and } R_{01} > R_{02}$$

must be statistically proved. Subscript 1 marks the upper straight line in Figure 4. In any event, the statistical t-test can be used for this purpose.

In our case, the divergent character in the first example was proved on 0.01 level of significance. Hence, the results of the first example as shown in Figure 2 should be interpreted as a substantial change in the aging rate, i.e., as a significant intervention into the rate of the pacemaker. (Let us remember that this result concerns D. melanogaster, i.e., a poikilothermal organism.)

Due to the higher level of statistical errors represented by broader limits shown by dashed lines in Figure 4, the corresponding statistical significance is on 0.05 level only. In spite of the lower mortality of mice exposed to 2-MEA, the results lead to the conclusion that the pacemaker was not influenced in the second case. While the first conclusion is probably not surprising, the second one might be. The generalization of this result will be possible after cautious verification by analysis of data of other experiments. Supposing for now that the result is confirmed, what conclusions may be drawn from it? If all premises are correct, there may be, for instance, something wrong in the intuitive equivalence between primary causes of aging on the subcellular level (which is iprobably the true nature of free radicals) and the general mechanism of the control of aging.

Let us briefly summarize the methodology from a practical point of view:

a) Divergent mortality curves indicate the changes in the rate of pacemaker, i.e., in the aging rate (see Figure 6).
b) A set of mortality curves with a single point of intersection is typical for non-age-related interventions (see Figure 7).
c) Parallel mortality curves and a set of crossing curves with multiple points of intersection have no unambiguous interpretation.

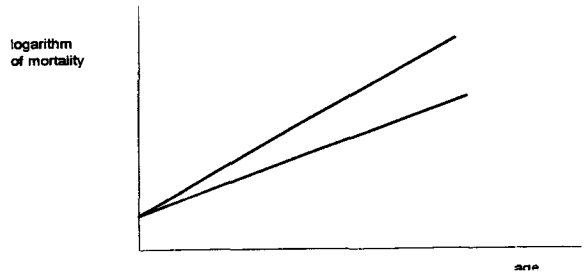

Figure 6: Relationship between mortality curves indicating changes in aging rate.

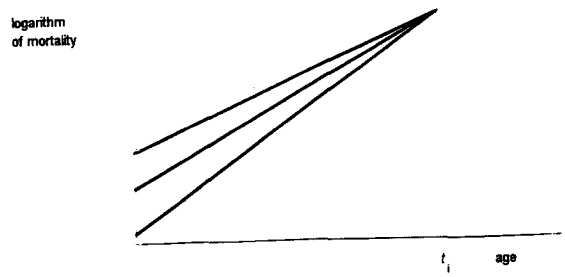

Figure 7. Mortality curves indicating no changes in aging rate.

As the suggested methodology is based on the validity of Gompertz law, only data from the Gompertzian region may be used for analysis. Just as in a human population, the linear course of the logarithm of mortality does not hold for very early and very late stages of life in many animal species. Fortunately, the typical region where Gompertz law holds covers, as a rule, a substantial part of the life span where the majority of all deaths occur (e.g., approx. 35 to 85 years for humans (16)).

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